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A specific profile of luteal phase progesterone is associated with the development of premenstrual symptoms

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Running title: Luteal phase progesterone profile and premenstrual symptoms

Abstract

There is a consensus that the development of premenstrual dysphoric states is related to cyclical change in gonadal hormone secretion during the menstrual cycle. However, results from studies seeking to link symptom severity to luteal phase progesterone concentration have been equivocal. In the present study we evaluated not only the absolute concentrations of progesterone but also the kinetics of the change in progesterone concentration in relation to development of premenstrual symptoms during the last 10 days of the luteal phase in a population of 46 healthy young adult Brazilian women aged 18-39 years, mean 26.5±6.7 years. In participants who developed symptoms of premenstrual distress, daily saliva progesterone concentration remained stable during most of the mid-late luteal phase, before declining sharply during the last 3 days prior to onset of menstruation. In contrast, progesterone concentration in asymptomatic women underwent a gradual decline over the last 8 days prior to menstruation. Neither maximum nor minimum concentrations of progesterone in the two groups were related to the appearance or severity of premenstrual symptoms. We propose that individual differences in the kinetics of progesterone secretion and/or metabolism may confer differential susceptibility to the development of premenstrual syndrome.

key words: premenstrual symptoms; progesterone; luteal phase; saliva

INTRODUCTION

Premenstrual syndrome (PMS) refers to a cluster of adverse psychological and physical symptoms experienced by many women in the late luteal phase of their menstrual cycle. The most commonly reported psychological symptoms included anger and irritability, mood swings/tearfulness, fatigue/lack of energy and food cravings, whilst physical symptoms include bloating, weight gain and breast tenderness (Dennerstein et al, 2011; 2012; Tschudin et al, 2010). Although the syndrome is not distinguished by a specific set of symptoms, there is a consensus that certain symptoms should be present for 2 or more days during the 14 days prior to menstruation, and subside by the end of the menstrual flow (Halbreich et al, 2007).

Premenstrual dysphorias are extremely common. In large surveys of European and Canadian women up to 90% of participants reported experiencing one or more symptoms. Up to 30% felt them to be considerably bothersome, whilst a smaller proportion of women (3-8%) who experienced severe and debilitating symptoms, were diagnosed with premenstrual dysphoric disorder (PMDD; American Psychiatric Association, 2013), which is considered a distinct psychiatric disorder (Halbreich et al, 2003; Tschudin et al, 2010; Wittchen et al, 2002). It is not clear however, whether PMS and PMDD are separate entities or whether PMDD is an extreme form of PMS. However, since the incidence of PMS is some 10-fold higher than PMDD; its effects are far more wide-reaching, due to the negative impact on family, friends and work colleagues as well as the individual herself. PMS and PMDD are undoubtedly multifactorial; at least three clinical subtypes have been recognised (Dennerstein et al, 2011; Freeman et al, 2011). Risk factors include high body mass index, stress, smoking, and early life emotional and physical abuse (Bertone-Johnson et al, 2010; 2014; Dennerstein et al, 2011). A genetic component is another contributory factor (Jahanfar et al, 2011).

Whilst it is well established that the menstrual cycle modulates the integration of emotional and cognitive processing in women (Hoyer et al, 2013), not all women develop premenstrual symptoms. In those who do, the main trigger factor is the cyclical production of sex hormones during the ovarian cycle (O'Brien et al, 2011). Apart from rare cases (O'Brien et al, 2011) premenstrual symptoms do not occur in anovulatory cycles (Muse et al, 1984; Hammarback et al, 1991) or following oophorectomy (Cronje et al, 2004). The luteal phase of the menstrual cycle, when premenstrual symptoms appear, is characterized by significant changes in secretion

of progesterone: production increases rapidly following ovulation and remains elevated throughout the luteal phase, before returning to basal levels prior to the onset of menstruation. Progesterone passes readily through the blood brain barrier (Pardridge et al, 1980). In post menopausal women (low stable progesterone concentration) administration of low doses of progesterone, which raised the concentration of the native steroid and its neuroactive metabolite allopregnanolone into the physiological range, induced negative mood whilst higher doses induced positive mood (Andréen et al, 2006; 2009).

Although we are not aware of comparable studies on women with PMS, several studies in PMDD sufferers have investigated the relationship between severity of symptoms and plasma concentration of progesterone during the luteal phase. The results from these studies have been equivocal, with reports of decreased (Rapkin et al, 1997; Wang et al 1996; Ziomkiewicza et al 2012), increased (Bäckström et al, 1983; Girdler et al. 2001; Hammarbäck et al, 1989; Redei and Freeman, 1993; Watts et al. 1985) or no difference (Hsiao et al, 2004; Rubinow et al. 1988) in luteal phase concentration of progesterone or its neuroactive metabolite allopregnanolone in women with PMDD versus asymptomatic controls. Interestingly, a more recent study has proposed an inverted U-shaped curve relationship between the severity of negative mood symptoms and allopregnanolone serum concentration (Bäckström et al, 2014).

In an effort to resolve this conundrum, we carried out a study in a population of healthy adult women in whom we made daily assessments of the presence of PMS-like symptoms during the luteal phase and the concentration of progesterone in the saliva. We considered not only the absolute concentrations of progesterone but also the kinetics of the change in progesterone concentration during the luteal phase. We found that neither maximum nor minimal concentrations of progesterone measured during the luteal phase could be linked to the appearance or severity of premenstrual symptoms. However, in the women who experienced significant symptoms of premenstrual distress, progesterone concentration remained stable during most of the luteal phase before declining sharply during the last 3 days. In contrast, progesterone concentration in asymptomatic women underwent a gradual declined over the final 8 days prior to menstruation.

METHODS

1.1 Participants

The sample was composed of healthy female volunteers, aged between 18 and 40 years, who were recruited from the local university population. The volunteers were invited through print ads, e-mail and social networks to participate in research into the menstrual cycle. Premenstrual dysphoric states were not mentioned. The local ethics committee approved the study (process number 8172/2014) and all participants signed a consent form. We included women who reported regular menstrual cycles and who had not used hormonal contraceptives in the last three months. Since the presence of other medical conditions might interfere with the evaluation of premenstrual symptoms, we excluded participants with a history of past diagnosis of severe mental illness, such as schizophrenia, bipolar disorder, major depressive disorder, obsessive-compulsive disorder, or who met criteria for any current psychiatric disorder. We also excluded women with a current use of psychoactive substances (except alcohol and tobacco), psychiatric medications or suspected pregnancy.

1.2 Clinical assessment

The absence of psychiatric diagnosis was confirmed by the application of the Structured Clinical Interview for DSM-IV (SCID) (First et al, 1997), no patient version, translated and adapted to Portuguese (Del-Ben et al, 2001). The menstrual cycle was characterized by means of a questionnaire specifically developed for this study, when information regarding the duration of the menstrual cycle in the last 3 months, the regularity of the menstrual cycles, and the date of the last period were registered. Screening and diagnostic interviews, and monitoring were performed by research assistants (nurses, psychologists) familiar with clinical research and properly trained in the assessment tools used in this study. These research assistants were also involved in the recruitment of participants, as well as telephone calls and face-to-face contacts, in order to ensure that the procedures for data collection were met properly. The next ovulatory date (Ov) for participants was estimated from the date of their last menstrual period (LMP) and the average duration of their menstrual cycles (MC) according to the following formula: Ov = LMP + MC - 14. If the interview happened between Ov and LMP, the volunteer was asked to contact the research assistants on the first day of her next period so that the Ov could be calculated. Participants were instructed to collect daily saliva samples and to complete symptom questionnaires (described below) during their luteal phase from the time of their estimated day of ovulation to the onset of menses or for 20 days, whichever was the shorter.

1.3 Premenstrual symptoms

The occurrence of premenstrual symptoms was measured by the short form of the Daily Record of Severity Problems Scale (DRSP, Endicott et al. 2006), translated into Portuguese. The DRSP is a 14-item self-report instrument, which accesses the presence of symptoms of common occurrence in the premenstrual period and their impact in the global functioning. The first 11 items of the DRSP are related to the following symptoms: depressed mood; anxiety; mood swings; irritability; less interest in usual activities; difficulty in concentration; lethargy; increased appetite; sleeping problems; feeling out of control; and physical symptoms. The 3 functional items evaluate the consequences of the reported symptoms on the quality/frequency of productivity, social activity and relationships. Each participant was directed to evaluate the occurrence of symptoms at the end of each day by applying a score on a 6-point scale: 1 = not at all, 2 = minimal 3 = mild; 4 = moderate, 5 = severe, 6 = extreme. Although initially designed to reflect DSM-IV criteria for PMDD, the DRSP can also be used to assess lesser degrees of severity of the premenstrual syndrome to track daily levels of severity of symptoms and impairment (Endicott et al, 2006). We recorded the occurrence of premenstrual symptoms daily, from the 14th day of the menstrual cycle based on the total score in the DRSP. As a reference point to assess the development of premenstrual symptoms we used the mean of the total score of the DRSP on the first two days of the analysis period i.e. in the early luteal phase 9 and 10 days prior to onset of menstruation. We defined symptomatic participants as individuals in whom the daily total DRSP score (sum of response to the 14 questions) increased >30% above the reference level in the early luteal phase on at least two successive days. Asymptomatic participants were defined as those in whom the daily total DRSP score did not vary by more than 30% at any time during the data collection period. No woman in this study met the criteria for premenstrual dysphoric disorder.

1.4 Salivary progesterone collection

The participants received a pack of collection aids and tubes for saliva collection (Salimetrics) pre-labeled with an identification code, and the date and time of collection. They also received written instructions about the procedures involved in saliva collection. They were told to perform the saliva collection at the time of awakening, before getting up and still in bed. They were asked to avoid drinking, eating and brushing teeth before saliva collection. From the 14th day of the menstrual cycle onwards, participants collected samples and stored the tubes in home freezers or in a domestic refrigerator, for up to 4 days. Previous studies have

shown that progesterone in saliva samples is unaffected by storage for at 4°C for 5 days. (Gröschl et al, 2001; Lewis 2006). The samples were collected from the participant's home by research assistants; then stored at -80°C in the laboratory prior to analysis.

1.5 Measurement of progesterone

Before the assays, samples were thawed to room temperature overnight, vortexed briefly and centrifuged at 10,000g for 5 min at 4°C in order to remove mucins and particulate matter from the saliva. 200µl samples of the supernatants were assayed for progesterone using commercially available ELISA kits (Cat. N° 1-1502 Salimetrics, State College, PA, USA) and following the procedures recommended by the manufacturer. Absorbance at 450nm was measured using a EL808 Microplate Reader (BioTek Instruments, Winooski, VT, USA) and the results were analysed using KC4 software, version 3.4. Intra-assay and inter-assay coefficients of variation were 9.2% and 8.3%, respectively. The sensitivity of the assays was 5.0 pg ml^{-1}.

1.6 Statistical analysis

Statistical analysis was performed using GraphPad PRISM v.6.0 software. Demographic and clinical characteristics were examined using Mann-Whitney or Fisher exact test. DRSP scores and salivary progesterone levels were analysed using T-tests, Friedman's test followed by Dunn's multiple comparisons or Spearman tests as appropriate. Extra Sum-of-squares F test was used for between group comparison of progesterone profiles.

The internal consistency of the DRSP was assessed by the Cronbach's alpha coefficient with alpha above 0.70 considered as a good index of internal consistency. (Chronbach, 1951). For this purpose, we considered the measures taken in the early luteal phase (days 10 and 9 prior to menstruation) and in the late luteal phase (days 1 and 0 prior to menstruation). Due to our small sample size, we were not able to carry out a factorial analysis. We therefore looked at the internal consistency of two sets of items of the DRSP: the 11 items related to premenstrual symptoms and the 3 items related to the impact of the symptoms on the global functioning.
The reliability of each item of the DRSP was tested by the Intraclass Correlation Coefficient (ICC), comparing the measures taken in the early luteal phase (day 10 vs. day 9) and in the late luteal phase (day 1 vs. day 0). Values between 0.40 and 0.75 are considered as a satisfactory agreement and values above 0.75 are considered as excellent (Fleiss, 1981).

RESULTS

2.1 Features of the sample

Sixty-three women were submitted to the screening interview. Eight participants were excluded from the study due to the use of antidepressants (n = 5), the diagnosis of hypothyroidism (n = 2), and the prescription, after the screening interview, of a hormonal contraceptive (n = 1). A further 9 participants, who were considered as eligible in the screening interview, declined to take part in the study after hearing about the procedures involved in data collection. Forty-six participants aged 18-39 years, mean 26.5±6.7 years were recruited into the study, which was carried out between June and October 2015. Data from 5 participants were excluded from the analysis due to incomplete data sets (n=3), progesterone below the level of detection of the assay (n=1) and in one case when the participant embarked on a course of antibiotic treatment during the study. In 6 participants menstruation had not occurred after 20 days of saliva collection. These women were suspected to have undergone atypical cycles and their data sets were therefore treated as a separate group and excluded from the main analysis.

In the participants who menstruated, collection periods ranged from 11-19 days, mean 14.6±0.4 days. Thus in order to have a complete data set for each participant we compared data from only the last 11 days before menstruation.
Good indices of internal consistency and reliability were obtained with Chronbach's coefficient in excess of 0.7 with respect to the total score, the subscale of premenstrual symptoms (items 1 to 11 of the DSRP) and the subscale of impact of the premenstrual symptoms on the global functioning (items 12 to 14) (Table 1). Similarly the reliability of each symptom, tested by the interclass coefficient, comparing the measures taken in the early luteal phase (day 10 vs. day 9 prior to onset of menstruation) and in the late luteal phase (day 1 vs. day 0 prior to menstruation) was in the range considered satisfactory or excellent (Table 2).

2.2 Premenstrual symptom scores v. salivary progesterone

Data was analysed from 35 participants who were cycling (i.e had menstruated within 20 days of the estimated date of ovulation). Subjective complaints measured by the DRSP scores showed a progressive increase during the luteal phase (Fig 1). During this period salivary progesterone concentration remained relatively stable during the

first 8 days of the collection period before declining rapidly over the last 3 days prior to menstruation (Fig 1).

2.3.1 Symptomatic and asymptomatic participants

Although the meand data from all participants indicated a progressive increase in PMS symptom scores during the luteal phase, there was considerable inter-individual variability. Inspection of individual data sets, considering an increase from the early luteal phase reference DRSP score of at least 30% as the criterion for PMS, revealed that two distinct subsets were present within the population: those who developed premenstrual symptoms (n = 22, 62.9%) and those who remained asymptomatic during the luteal phase (n = 13, 37.1%). No significant differences between symptomatic and asymptomatic participants were found regarding age (Mann-Whitney p = 0.618), marital status (Fisher exact test, p = 0.618) and years of education (Fisher exact test, p = 0.524).

2.3.2. Symptomatic participants

In terms of individual symptoms, the most common complaints (moderate intensity or worse, score 4 or above on two or more days) were: felt nervous or irritated (55%); slept more; took naps; felt it more difficult to rise when needed; had trouble sleeping and suffered insomnia (45%); felt anxious, tense or restless (40%); had mood swings (eg felt suddenly sad or tearful) or was more sensitive to rejection or easily hurt feelings (40%); increased appetite, ate more or felt compulsion for specific foods (40%). In addition, 80% of participants who experienced negative psychological symptoms also reported being troubled by physical symptoms such as pain or breast enlargement, feeling of being bloated, weight gain, headaches, joint or muscle or other physical symptoms.

In the symptomatic group the DRSP score increased steadily during the 11 days of data collection during the luteal phase and was significantly higher, compared to the first sample day, during the last 6 days prior to menstruation (Fig 2A, $p<0.05$ Friedman test followed by Dunn's multiple comparisons). Analysis of salivary progesterone in the symptomatic participants over the same period prior to menstruation showed that progesterone concentration remained relatively stable during early/mid luteal phase before showing a sharp decline during the last 3 days prior to menstruation (Fig 2A). Thus paradoxically, DRSP scores increased when progesterone concentration was stable and continued to rise further as progesterone concentration underwent a sharp decline (Fig 2A).

2.3.3. Asymptomatic participants

In the asymptomatic participants (37.1%, n=13), DRSP scores did not change significantly during the collection period (Fig 2B), whereas salivary progesterone concentration underwent a gradual linear decline (Fig 2B). The linear decline in progesterone concentration was not a 'smoothing effect' due to a misalignment of peaks in some subject cancelling peaks in others. When the dataset was re-analyzed with the peaks aligned, the progesterone profile was still best represented as a straight line (*9y = -9.6746x + 237.91).*

2.3.4. Comparison between symptomatic and asymptomatic participants

The mean length of the luteal phase (number of days between estimated ovulation and onset of menstruation) was similar in symptomatic and asymptomatic participants (14.6±0.6 vs. 14.6±0.56 days respectively, p=0.5, unpaired T-test). However, the DRSP score at the beginning of the data collection period was significantly higher in the asymptomatic group than in the participants who went on to develop premenstrual symptoms (29.0±2.8 *v.* 21.1±1.1 respectively, p<0.01). The salivary progesterone concentration on the first collection day for analysis (10 days prior to menstruation) was similar in the two groups (176.1±24.3 *v.* 207.8±32.9 pg/ml respectively, p=0.23, unpaired test). However, although the progesterone concentration on the final collection day (first day of menstruation) had declined to similar levels in both symptomatic and asymptomatic participants (147.2± 18.4 *v.* 129±22.7 pg ml-1, p>0.05, unpaired T-test), there was a marked difference in the time course of the change in progesterone concentration between the two groups. In symptomatic participants the time course was best described by the third order polynomial function $y=124.5 - 44.49x - 7.548x^2 - 0.3636x^3$ (Extra Sum-of-squares F test, with a first order polynomial (linear) as the null hypothesis, *v.* a 3rd order polynomial p=0.026) (Fig. 2A), In marked contrast, progesterone concentration in the asymptomatic group showed a linear decline in over the same period (Fig 2B) (extra sum of squares F-test, p=0.8, do not reject null hypothesis). At the end of the luteal

phase, 1-3 days prior to onset of menstruation at day 0, the decline in progesterone concentration in women who developed symptoms of PMS followed a steep linear trajectory (r^2=0.99). The slope of the regression line fitted to the data during this period was significantly steeper than the slope for the participants who remained asymptomatic (P<0.05, Extra Sum of Squares F-Test).

2.3.5. Participants with atypical cycles (n=6)

Although we initially excluded from the main data set those participants who did not menstruate within 20 days of their estimated date of ovulation, it proved instructive to examine their salivary progesterone concentration and DRSP scores. In this group the basal DRSP scores on the first day of data collection were significantly higher than the participants who menstruated (37.7±5.8 v. 24.0±1.6, P=0.02, T-test); moreover the scores remained stable at this high level throughout the data analysis period (Fig 3). However, mean salivary progesterone concentration at the start of the analysis period was not significantly different from the women who menstruated (124.8±37.7 *v.* 187.8±23.8 pg ml^{-1} for all symptomatic and asymptomatic participants, P=0.1, T-test). Moreover, the participants who failed to menstruate within 20 days of their estimated date of ovulation did not show a decline in progesterone during the collection period (Fig 3), suggesting that these individuals might have been undergoing atypical cycles.

DISCUSSION

Within our cohort of young healthy Brazilian women 63% reported experiencing premenstrual symptoms, which increased in severity over the 10 day long data collection period preceding the onset of menstruation. This incidence of premenstrual symptoms is in line with reports on women in other developed countries (Campbell et al, 1997; Hylan et al, 1999; Johnson et al, 1988). No woman in our study satisfied the criteria for PMDD.

Collection of daily saliva samples offers an attractive non-invasive and stress-free means to obtain repeated measurements of progesterone (Lewis, 2006). In the present study the mean salivary progesterone concentration of all participants (excluding those with atypical cycles) at the beginning of our data analysis period (10 days prior to onset of menstruation) was similar to that reported during the early

luteal phase using ELISA (Gandara et al, 2007). Higher values have been reported using radio-immunoassay (Chatterton et al, 2005; Celec et al, 2009), and may reflect a differential sensitivity between the two forms of assay. Salivary progesterone concentration remained essentially stable during the mid-late luteal phase before falling during the last 3 days prior to menstruation. A similar salivary hormonal profile in during the mid-late luteal phase was reported by Ishikawa et al (2002) and also by Ziomkeiwicz et al (2012) in women who showed high luteal phase aggression/irritability. However, as noted previously by others (Gann et al 2001; Gandara et al 2007), there was considerable between-subject variability, particularly in relation to the time course of changes in progesterone concentration over the luteal phase collection period. The factors contributing to the variability in these studies were not investigated.

In the present study salivary progesterone in asymptomatic women, who showed no significant change in subjective complains of premenstrual symptoms during the luteal phase, underwent a steady linear decline over the final 10 days leading up to menstruation. It seems unlikely that these women failed to ovulate. If they had not ovulated, their level of progesterone secretion would be expected to be very low in the absence of a corpus luteum, In fact, their peak progesterone concentration at the beginning of the collection period did not different significantly from the women who developed symptoms of PMS. Moreover, their early luteal phase progesterone was similar to that reported previously using an ELISA assay, in saliva of cycling women (Gandara et al 2007). In the women in the present study who developed symptoms of PMS, progesterone concentration at the beginning of the data collection period was similar to the asymptomatic participants, but remained stable at this level until 3 days prior to menstruation, when it declined rapidly. This profile is very similar to that reported for plasma luteal phase progesterone concentration in women with PMDD (Andréen et al, 2006).

At first sight the data from the participants who developed symptoms of PMS appear anomalous since premenstrual symptom scores increased during the period when progesterone was stable and then increased even further when progesterone secretion declined rapidly at the end of the cycle. In animal models anxiolytic and even sedative effects of progesterone and its neuroactive metabolites have been widely reported following acute administration (Bitran et al, 1991; 1995; Fernandez-Guasti and Picazo, 1992). However, it is worth noting that at low concentrations, which are more likely to produce plasma concentration within the physiological

range, these steroids can be anxiogenic or induce aggressive behaviour (Miczek et al, 2003; Gulinello and Smith, 2003).

Adverse effects of progesterone on mood may also be exacerbated on chronic exposure. In post menopausal women, who have a low level of endogenous progesterone, 14 days of repeated administration of progesterone designed to produce a stable physiological concentration of the steroid, was associated with a progressive worsening of negative mood (Andréen et al, 2006). This finding is analogous to the situation in the present study where in participants who developed PMS, the symptom score progressively increased during the mid-luteal phase when their progesterone concentration was stable i.e. **before** the rapid fall in the days prior to menstruation. Paradoxically, their symptoms continued to worsen when progesterone underwent a sharp decline prior to onset of menstruation. In rats, withdrawal from chronic dosing with exogenous progesterone has been reported to precipitate raised anxiety and increased responsiveness to acute mild psychological stress (Smith et al, 1998; 2006; Devall et al, 2009). This effect was shown to be mediated not by the native hormone, but by its neuroactive metabolite allopregnanolone (Smith et al, 1998). The withdrawal of the steroid precipitated upregulation of α4, β and δ subunits of the $GABA_A$ receptor, which was associated with increased excitability in brain areas containing nerve circuits associated with mediating responsiveness to acute stress (Devall et al, 2009; 2015; Smith et al, 1998; 2006). Similar effects were reported in spontaneously cycling rats during the late diestrus phase of the estrous cycle (Devall et al, 2009; 2015), when progesterone declines rapidly but estrogen secretion remains relatively stable (Butcher et al, 1974). Interestingly, a more recent study on women has proposed that $GABA_A$ receptor dysfunction may be linked to susceptibility to developing PMS, based on differences in sensitivity to allopregnanolone during the luteal phase in women with premenstrual dysphoric disorder (PMDD) compared to asymptomatic individuals (Timby et al, 2016). A similar dysfunction may be present in women who develop PMS.

The above findings suggest that two mechanisms could come into play sequentially during the luteal phase in women who developed symptoms of PMS. The initial increase in symptom score during the early/mid luteal phase may result from continued exposure to relatively stable progesterone concentration, followed by further exacerbation of symptoms triggered by a withdrawal effect as progesterone secretion declines in the late luteal phase. Interestingly, the rate at which

progesterone declines appears to be an important determinant of the drug withdrawal effect. In a rat model, adverse behavioral changes were precipitated only following a rapid withdrawal from long term dosing with progesterone, but did not occur if progesterone declined slowly (Doornbos et al, 2009). The latter scenario is analogous to the asymptomatic participants in the present study who experienced a gradual decline in progesterone during the mid and late luteal phase and who did not develop premenstrual symptoms.

A limitation to our study is the absence of follicular phase concentration of progesterone for each participant. This would have provided unequivocal evidence of ovulation. However, in the participants who menstruated before the end of the 20 day collection period (mean onset of menstruation 14.6 days after ovulation), peak progesterone concentration was commensurate with the expected values for the luteal phase using our assay system (see below). These two factors (menstruation and normal luteal phase progesterone) give us confidence that they were cycling normally. In a similar vein, the absence of DSRP scores for the follicular phase did not allow us to determine whether, in our presumed asymptomatic group in whom DSRP scores remained stable during the collection period, PMS symptoms had developed very early post ovulation and remained constant throughout the luteal phase. This seems an unlikely scenario however. Our participants scored from 1 (no symptoms) up to 6 (severe) on the 14 point DSRP questionnaire. A score of 14 indicates no symptoms at all, whereas a score of 28, which is the mean early luteal phase score for our asymptomatic group, would indicate on average only mild symptoms. On the other hand our symptomatic subjects' mean scores rose steadily by 75% to peak in the late luteal phase, consistent with development of PMS.

In the present study 18% of our participants had not menstruated by 20 days after their estimated date of ovulation, and were instructed to cease data collection. There was no evidence of cyclicity in salivary progesterone concentration in this group and the participants also failed to report any significant change in premenstrual symptoms during the data collection period. A similar incidence of abnormal progesterone profiles has been reported previously and was presumed to reflect anovulatory cycles, (Gandara et al 2007) and is in line with the earlier finding in women with PMDD that symptoms do not appear during anovulatory cycles (Hammarbäck et al 1991). However in our participants who did not menstruate, the progesterone concentration was in the range expected during the luteal phase, according to the manufacturer's guidelines for the assay kits we used

(www.salimetrics.com/assets/documents/1-1502.pdf). It is possible that the failure to menstruate reflected an exceptionally long luteal phase in these women. However, even after 20 days of sample collection, there was no sign that progesterone concentration was starting to decline (Fig 3), which would be expected as a prelude to menstruation.

An interesting feature of this group was that their DRSP scores at the beginning of the collection period were very high (higher than the maximum reached by the symptomatic group) and remained so throughout the collection period. In rats (males and females), we found that exposure to restraint stress evoked a parallel increase in secretion of both progesterone and corticosterone from the adrenal cortex (Kalil et al, 2013). Very recently, a parallel stress-induced increase in secretion of cortisol and progesterone has also been reported in humans (men and women) (Juster et al, 2016). It is possible that the high DRSP scores in our participants who did not menstruate were a response to personal stress or other distress at the time of the study, which stimulated adrenal secretion of progesterone and interfered with the participants' normal menstrual cycle. Regardless of the underlying source of progesterone in these women, the coincident high stable progesterone and high stable DSRP scores in this group are interesting because they emphasize that the dynamic of the progesterone profile during the luteal phase, rather than the absolute level achieved appears to be the key factor that precipitates worsening of premenstrual symptoms. A significant increase in severity of premenstrual symptoms developed only when progesterone concentration changed abruptly.

The results of the present study suggest that individual differences in secretion or metabolism of progesterone during the luteal phase could be determinants of susceptibility to developing negative mood symptoms. The critical factor may not be the absolute concentration of progesterone reached during the luteal phase but rather, the rate at which progesterone declines. The factors that determine individual differences in the rate of secretion and/or metabolism progesterone are not clear. Progesterone is metabolised to its neuroactive metabolite allopregnanolone via the actions of two enzymes: 5α-reductase and 3α-hydroxysteroid reductase. Interestingly, the presence of a polymorphism in the cytosine/cytosine (C/C) genotype for SRD5A1SNP, rs501999, the gene that encodes for type I 5α-reductase, appears to protect women against developing severe premenstrual symptoms (Adams and McCrone, 2012). Moreover mice deficient in Type 1 5α-reductase did not show the estrous cycle-linked changes in affective behavior displayed by wild

type animals (Koonce et al, 2012). These results suggest that further investigation of the activity of Type 1 5α-reductase in relation to metabolism of progesterone and susceptibility to PMS is warranted.

Although this study focused on the effect of progesterone and its metabolite allopregnanolone, it must be emphasized that these are not the only neuroactive steroids to change during the menstrual cycle. Although progesterone is the dominant steroid in the luteal phase secretion of estrogen also fluctuates. Estrogen can act as a modulator of synaptic plasticity, connectivity, and cognitive behaviors in its own right, as well as modulating the effects of progesterone to influence behavior during the ovarian cycle (Llaneza and Frye, 2009; Srivastava and Penzes, 2011). It is not possible to determine from the present findings, the extent that estrogen may have contributed to the development of symptoms of PMS.

CONCLUSIONS

The present study suggests that the rate of change in progesterone concentration during the luteal phase of the cycle could be an important determinant of susceptibility to developing the adverse psychological reactions, which characterize PMS. However, progesterone should not be considered in isolation. Although beyond the scope of the current study, consideration should be given to the influence of estrogen as well as progesterone in the development of premenstrual dysphorias.

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Fig 1. Pooled data from all participants (n=35) to show time course score of mean PMS measured by the Daily Record of Severity Problems Scale (DRSP) total score and saliva progesterone concentration over the 11 days prior to menstruation. **p<0.01; *** P<0.001 compared to Day -10, Friedman test across all days followed by Dunn's multiple comparisons.

Fig 2 A. Time course of mean PMS score and saliva progesterone concentration in 22 symptomatic women over the 10 days prior to menstruation. ** p<0.01; *** compared to Day -10, Friedman test across all days followed by Dunn's multiple comparisons.
B. Time course of mean PMS score and saliva progesterone concentration in 12 asymptomatic women over the last 10 days prior to menstruation. Friedman's test across all days followed by Dunn's test for multiple comparisons revealed no significant differences. Lines of best fit were determined by extra sum of squares F-test (GraphPad PRISM).

Fig 3. Time course of mean PMS score and saliva progesterone concentration in 6 women had not started to menstruate 20 days post their calculated day of ovulation. Friedman's test across all days followed by Dunn's test for multiple comparisons revealed no significant differences. Lines of best fit were determined by extra sum of squares F-test (GraphPad PRISM).

Table 1: Internal consistency (Cronbach's Alpha) of the DRSP, according to the total score and the subscale of premenstrual symptoms (items 1 to 11 of the DSRP) and the subscale of impact of the premenstrual symptoms on the global functioning (items 12 to 14).

	Day 10	Day 9	Day 1	Day 0
Total score	0.881	0.912	0.926	0.911
Premenstrual Symptoms	0.865	0.881	0.916	0.899
Global functioning	0.888	0.854	0.841	0.863

Table 2: Intraclass Correlation Coefficient (ICC) of the items of the DRSP applied in the early luteal phase and in the late luteal phase.

	Item of the DRSP	Early luteal phase (day 10 vs. Day 9)	Late luteal phase (day 1 vs. day 0)
1	Depressed mood	0.547	0.519
2	Anxiety	0.687	0.743
3	Mood fluctuations	0.773	0.771
4	Irritability	0.839	0.696
5	Anedhonia	0.532	0.847
6	Concentration	0.757	0.832
7	Tiredness	0.596	0.828
8	Changes in appetite	0.849	0.89
9	Sleep disturbances	0.627	0.708
10	Loosing control	0.607	0.677
11	Physical symptoms	0.858	0.643
12	Impairment at work/school	0.522	0.78
13	Impairment of social activities	0.759	0.669
14	Impairment in relationships	0.705	0.708

Table(s)

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Figure(s)
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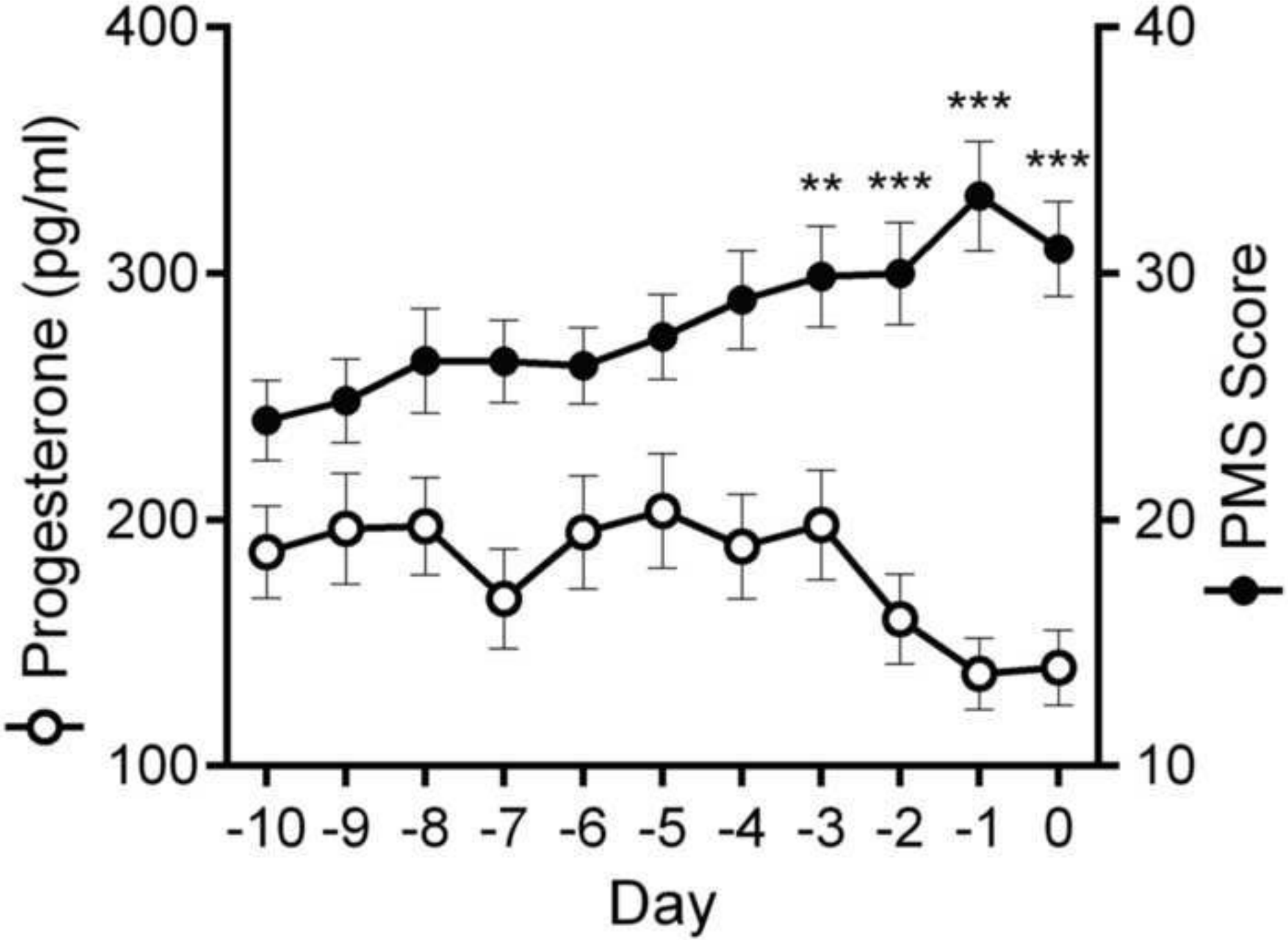

Figure 2

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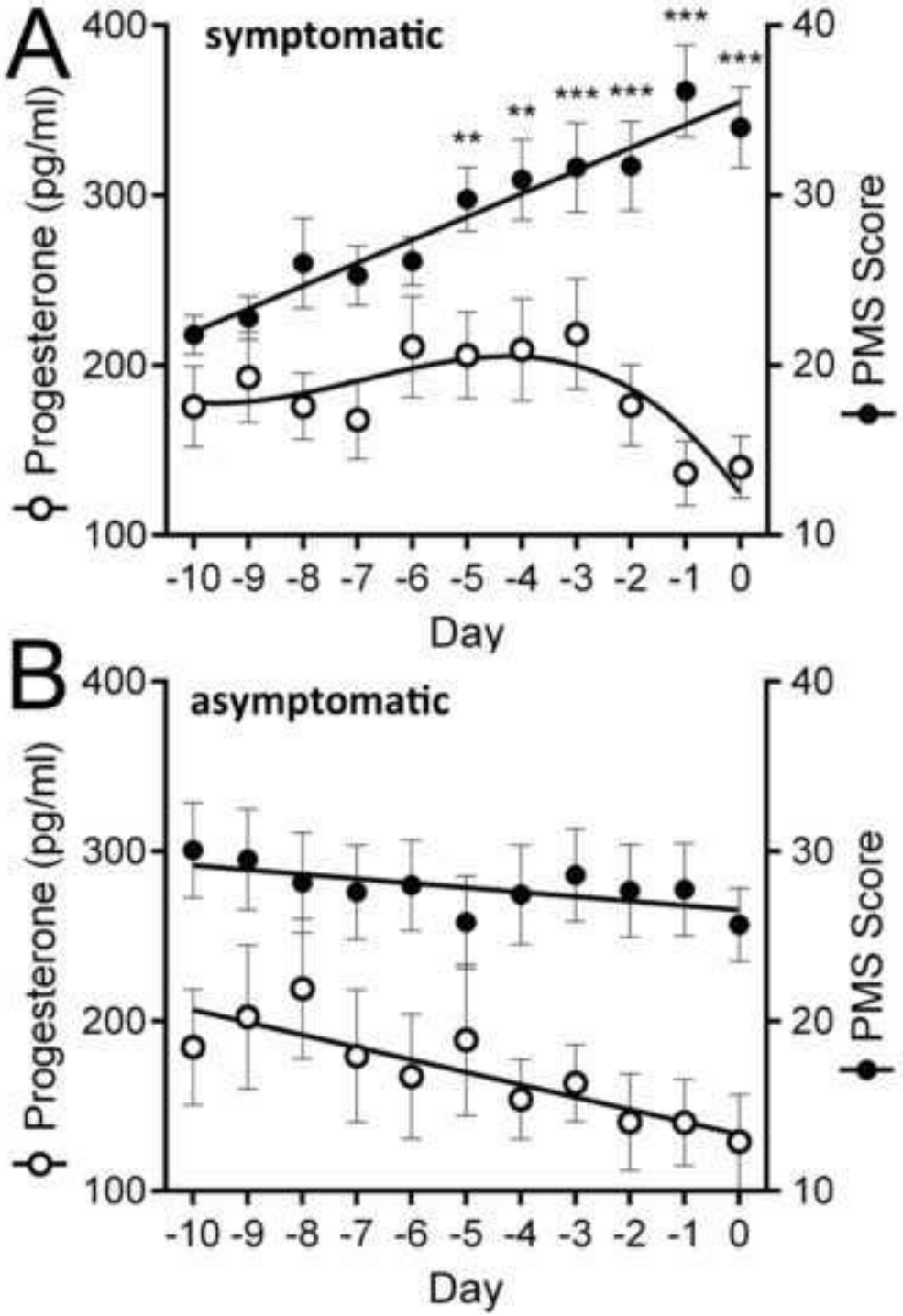

Figure 3
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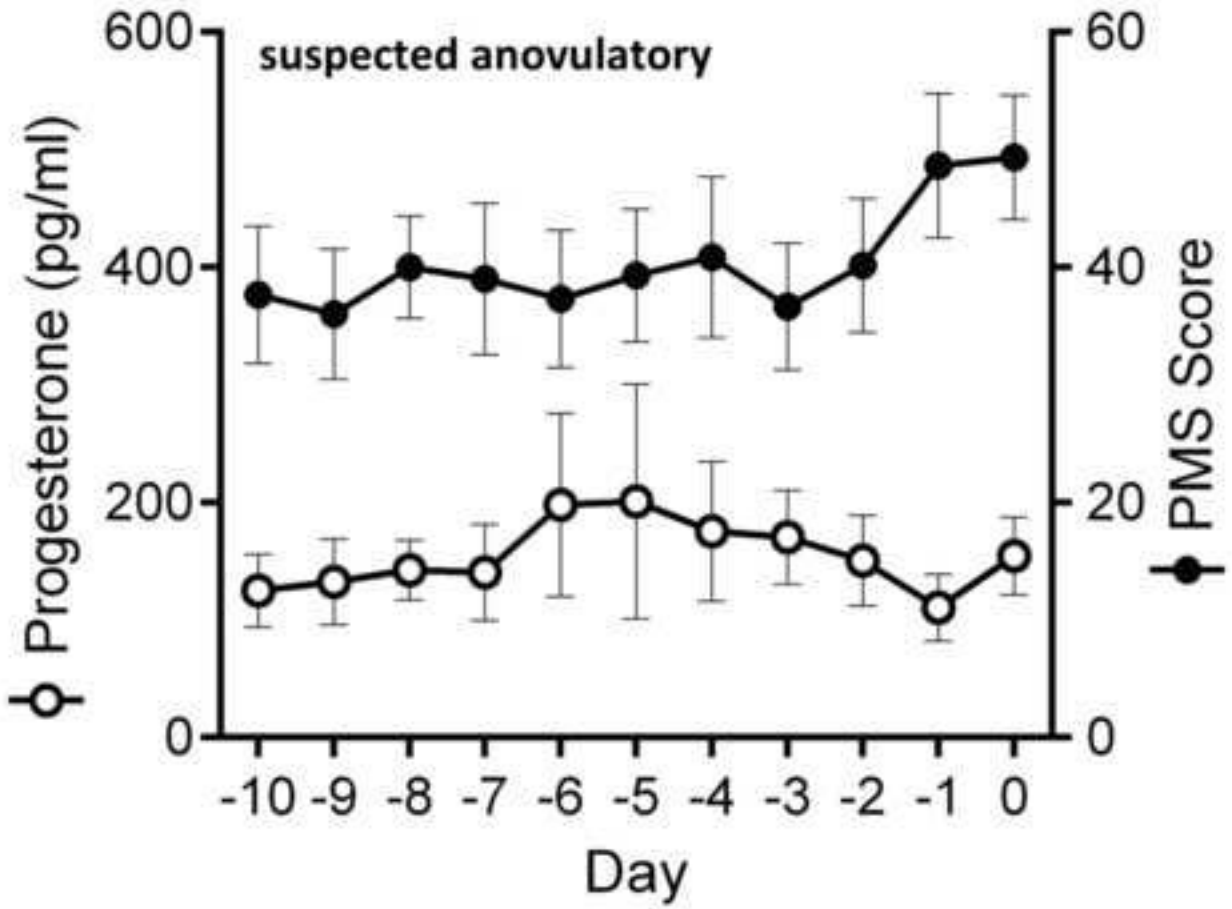

***Contributors**

TAL conceived the study, obtained funding (with MLB), contributed to study design, analysed the data and wrote the manuscript.
JAAF contributed to the study design and managed progesterone measurement.
VGG contributed to the experimental design and study protocol, obtained ethical approval, supervised data collection, collaborated in the interpretation of the data.
MCMF supervised data collection, collaborated in the interpretation of the data.
CML was in charge of the acquisition of data, training of research assistants and collection and storage of the samples, and collaborated in data analysis.
CMD-B contributed to the experimental design and study protocol, obtained ethical approval, supervised data collection, collaborated in the interpretation of the data and critically revised the manuscript.
MLB obtained funding, managed data collection, contributed to data analysis and preparation of the manuscript.

Women with sharp decline in luteal phase progesterone developed premenstrual symptoms

Women with gradual decline in luteal phase progesterone had no premenstrual dysphoria

Rate of progesterone metabolism linked to development of premenstrual dysphorias

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***Conflict of Interest**

None of the authors expresses a conflict of interest

***Role of the Funding Source**

None of the funding agencies had any input to the study design; the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.